

Ambient sulfur dioxide could have an impact on testicular volume from an observational study on a population of infertile male

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Abstract

Background

The present retrospective study investigates the effect of environmental pollutants on male reproductive health.

Methods

Male patients with primary infertility (n = 282) from a single center (National Taiwan University Hospital, Taipei, Taiwan), between January 2016 and December 2017, were identified. Patients were physically examined for the presence of varicocele, and the volume of both testicles. Semen quality was measured in terms of total sperm count (millions), sperm concentration (millions/ml), and the percentage of motile sperm cells and the sperm cells with normal morphology. Data on the concentration of SO₂ (ppb), O₃ (ppb), NO and NO₂ (ppb), and PM_{2.5} concentrations (μg/m³), measured on daily and hourly basis, were acquired from the Environmental Protection Administration Executive Yuan, Taiwan. Individual exposure to pollutants was estimated based on the reported residential address of patients. Statistical analysis indicated the impact of each pollutant on the testicular volume and semen parameters.

Results

Mean ± SD of age was 36.7 ± 7.3 years. The average sperm count and concentration was 41.9 million/ml and 34.1 million/ml, respectively. Except for PM_{2.5}, the mean concentration of all the particulate matters were within the reference value. NO₂ and SO₂ exposure were negatively associated with the sperm concentration and motility, and testicular volume, respectively.

Conclusions

NO₂ and SO₂ exposure were negatively associated with the sperm concentration and motility, and testicular volume, respectively, in a population of infertile male. Nevertheless, studies are needed to ascertain the impact of different exposure levels of pollutants on the semen parameters.

Background

The effects of changes in environmental factors on reproductive health is increasingly concerning. Exposure to chemicals such as perchloroethylene, hydrocarbons, and chemical ambient pollutants negatively affects sperm quality and, particularly, sperm morphology.¹⁻³ Several studies have investigated the effects of lifestyle factors such as alcohol and smoking exposure on reproductive health.⁴⁻⁷ However, the results were inconsistent, which may be explained by the small sample size and difficulty in quantifying the exposure to tobacco and alcohol; moreover, most studies were retrospective, and therefore, the effects of confounding factors and bias could not be avoided.

Recently, the effects of environmental toxicants, such as particulate matters of size 2.5 μm (PM2.5), nitrogen oxides, sulfur oxides, and carbon monoxide, on human reproductive health has gained increasing attention. Nieuwenhuijsen et al. reported that traffic-related air pollution reduces fertility rates.⁸ Additionally, using a regression model that estimated air pollution concentration, Frutos and colleagues demonstrated a negative correlation between air pollutants, such as particulate matters and nitrogen oxides, and fertility.⁹

In addition to altered fertility rates, Farhat et al. discovered an association between ozone and decreased sperm quality, especially the sperm count in patients diagnosed with systemic lupus erythematosus.¹⁰ The effects of ambient pollutants on animals were demonstrated using murine models. Both fluoride and sulfur dioxide, which exist in the atmosphere, showed negative effects on the sperm quality of male mice.¹¹ Additionally, PM2.5, a worldwide concern, increases oxidative stress in the gonad, as observed through altered expression levels of proinflammatory cytokine genes.¹² Furthermore, the testicular biopsy of mice exposed to a high PM2.5 level showed few mature sperms, indicating developmental arrest.¹³

However, limited studies have investigated the direct effects of environmental chemical particles, including sulfur oxides, nitro oxides, and ozone, on semen quality and the male reproductive system. We focused on the association between environmental pollutants and male reproductive health.

Methods

We analyzed data of male patients diagnosed with primary infertility from a single center (National Taiwan University Hospital, Taipei, Taiwan) from January 2016 to December 2017. Primary infertility was defined as the inability to conceive under an unprotected sexual encounter in ≥ 12 months, as defined by the World Health Organization (WHO).¹⁴

Physical examination included determining the presence and grading of varicocele and recording the volume of both testicles. The testicular volume was estimated using the Prader orchidometer (Bayer, Müllerstraße 178, Berlin, Germany). Genital malformation, if any, was recorded.

Body weight, height, basic blood test, sperm analysis, and sex hormone profiles were examined at the clinic. In addition, lifestyle habits, including smoking and alcohol consumption, were recorded.

Semen analysis

Patients were asked to practice abstinence for 3–5 days before semen collection. Each man was provided with a wide-mouth plastic container. The semen sample was collected through masturbation and was sent to the laboratory at our institute. Each sample was smeared, stained, and preserved. The following semen variables were used as outcomes: total sperm count (million), sperm concentration (million/ml), percentage of motile sperm cells, and the percentage of sperm cells with normal morphology.

Ambient pollutant data and analysis

Data were acquired from the Environmental Protection Administration Executive Yuan, Taiwan (<https://airtw.epa.gov.tw>). Air pollutants inclusive of Sulfur dioxide (SO₂), Ozone (O₃), and Nitrogen oxides (NO_x), including nitrogen monoxide (NO) and nitrogen dioxide (NO₂), and particulate matters 2.5 (PM_{2.5}) concentrations were automatically recorded at 78 monitoring stations throughout Taiwan. The data collected from these stations were recorded automatically using equipment, and the measures were previously validated manually by checking concentrations through linear regression. The concentration of each pollutant was continuously measured on daily and hourly bases. The monthly average of each pollutant was calculated if ≥ 20 daily average records were available for a month. In this study, individual exposure to air pollution was estimated on the basis of current residential addresses reported by the study participants and the mean concentrations of air pollutants in the study period (January 2016 to December 2017).

Statistical analysis

Values were expressed as mean \pm standard deviation. Linear regression with a regression coefficient β and a 95% confidence interval was applied to indicate the effect of each unit of pollutant on the testicular volume and semen parameter. Smoking and medical comorbidity, including obesity and the status of varicocele, were adjusted in the multivariate regression model. We set the significance level at $p < 0.05$. All data analyses were performed using SPSS version 12 (SPSS Inc., Chicago, IL, USA).

Results

The demographic characteristics of the included participants are summarized in Table 1a and 1b. During the study period, 282 participants residing in Taiwan were included. The mean (\pm standard deviation) age, body mass index (BMI), and the size of the right and left testes of all the participants were 36.7 ± 7.3 years, 24.6 ± 4.2 kg/m², and 14.18 ± 2.7 and 14.30 ± 2.4 ml, respectively. In total, 64 participants (22%) had varicocele, and the majority of the participants ($n = 217$, 77%) were nonsmokers. The majority of the participants ($n = 265$, 93%) resided in northern Taiwan; 3%, 3%, and 1% of the patients were from central, southern, and other parts of Taiwan, respectively.

Table 1

a. Demographic characteristics of the study participants

| Characteristics | Mean \pm standard deviation | Range |
|-----------------|----------------------------------|---------|
| Age | 36.7 ± 7.3 years | 19–69 |
| BMI | 24.6 ± 4.2 kg/m ² | 17.3–36 |
| Right testis | 14.18 ± 2.7 ml | 6–20 |
| Left testis | 14.30 ± 2.4 ml | 5–20 |

Table 1

b. Demographic characteristics of the study participants

| Characteristics | N, total = 282 | % |
|--|-------------------------------|----------|
| Cigarette | | |
| Smoker | 65 | 23 |
| Nonsmoker | 217 | 77 |
| Varicocele | | |
| Present | 64 | 22 |
| Absent | 218 | 78 |
| Residency | | |
| Northern Taiwan | 265 | 93 |
| Central Taiwan | 7 | 3 |
| Southern Taiwan | 7 | 3 |
| Other | 3 | 1 |
| Table 2. Distribution of sperm parameters | | |
| Sperm parameters | Mean \pm standard deviation | Range |
| Sperm count | 41.9 \pm 56.6 mln | 0.25–280 |
| Sperm concentration | 34.1 \pm 38.8 mln/ml | 0.1–420 |
| Sperm motility | 30.5% \pm 16% | 0–75 |
| Normal form | 23.6% \pm 9.2% | 0–80 |

Sperm parameters

The average sperm count was 41.9 ± 56.6 million, and the average concentration was 34.1 ± 38.8 million/ml. The total sperm motility was $30.5 \pm 16\%$, and the average normal form was $23.6 \pm 9.2\%$ (Table 2). The mean values of the total sperm count, concentration, and normal form were higher than the cut-off values according to WHO standards.¹⁵ However, the percentage of the progressively motile spermatozoa was low.

| Table 2. Distribution of sperm parameters | | |
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| Normal form | 23.6% ± 9.2% | 0–80 |

Air pollution levels

The average levels of PM_{2.5}, O₃, SO₂, and NO_x were 18.0 µg/m³ (range = 10.9–27.2 µg/m³), 27.3 ppb(parts per billion) (range = 21.0–40.6 ppb), 2.9 ppb (range = 1.7–5.6 ppb), and 29.0 (range = 3.7–91.9 ppb), respectively. According to WHO air quality guidelines, the recommended annual mean for PM_{2.5}, O₃, SO₂, and NO₂ were 10 µg/m³, 100 ppb, 20 ppb, and 40 ppb, respectively.¹⁶ Except for PM_{2.5}, the mean concentrations of particulate matters in Taiwan were within the reference values. The eastern part of Taiwan, which consists of mostly rural regions, had low SO₂, NO_x, and PM_{2.5} levels. The distribution of ozone did not considerably vary between the different parts of Taiwan, but a high O₃ level was observed in Kinmen, an island located on the east coast of China. All ambient pollutants were present in the highest level in the southern part of Taiwan, including Tainan, Kaohsiung, and Kinmen, in all seasons. The PM_{2.5} level was high in winter and low in summer, and the regional concentration difference of PM_{2.5} was particularly obvious in January and February. (Fig. 1 to 5)

Tables 3 and 4 summarize multiple regression analysis results for the semen parameter and testicular volume after adjusting for blood sugar, BMI, varicocele, and smoking. The exposed mean concentrations of nitrogen oxides in the study period were negatively associated with sperm concentration and total motility ($p < 0.05$). No statistically significant relationship was observed between exposure to other air pollutants and sperm parameters. The testicular volume was negatively associated with the exposed mean concentration of SO₂ ($p < 0.01$).

Table 3
Multivariable linear regression by using a sperm parameter for
exposure to ambient pollutants

| Sperm parameters | Regression coefficients (95% confidence interval) | p value |
|-------------------------|--|----------------|
| Sperm count | | |
| PM2.5 | 0.896 (- 16.113 to 40.225) | 0.382 |
| SO ₂ | -0.891 (- 169.427 to 68.260) | 0.384 |
| NOx | -1.136 (- 12.614 to 3.738) | 0.270 |
| O ₃ | -0.196 (- 18.947 to 15.698) | 0.846 |
| Sperm concentration | | |
| PM2.5 | 0.109 (- 13.821 to 15.340) | 0.914 |
| SO ₂ | -1.302 (- 99.796 to 23.235) | 0.208 |
| NOx | -2.174 (- 8.627 to - 0.164) | 0.043* |
| O ₃ | -1.749 (- 16.458 to 1.475) | 0.096 |
| Sperm motility | | |
| PM2.5 | -0.303 (- 7.508 to 5.608) | 0.765 |
| SO ₂ | 0.049 (- 27.026 to 28.308) | 0.962 |
| NOx | -2.321 (- 4.014 to - 0.207) | 0.032* |
| O ₃ | -1.248 (- 6.437 to 1.624) | 0.227 |
| Sperm normal form | | |
| PM2.5 | 1.248 (- 3.566 to 13.322) | 0.234 |
| SO ₂ | -0.617 (- 46.216 to 25.681) | 0.548 |
| NOx | 1.400 (- 1.147 to 5.369) | 0.185 |
| O ₃ | 0.992 (- 4.546 to 12.267) | 0.339 |
| *p < 0.05 | | |

Table 4
Multivariable linear regression by using the testicular volume for exposure of
ambient pollutant

| | Regression coefficients (95% confidence interval) | p value |
|-----------------|---|---------|
| Right testicle | | |
| PM2.5 | -0.472 (- 0.956 to 0.607) | 0.643 |
| SO ₂ | -3.101 (- 8.279 to - 1.575) | 0.006* |
| NO _x | -0.967 (- 0.376 to 0.140) | 0.347 |
| O ₃ | -1.337 (- 1.007 to 0.226) | 0.199 |
| Left testicle | | |
| PM2.5 | 0.472 (- 0.956 to 0.607) | 0.643 |
| SO ₂ | -3.101 (- 8.279 to - 1.575) | 0.006** |
| NO _x | -0.967 (- 0.376 to 0.140) | 0.347 |
| O ₃ | -1.337 (- 1.007 to 0.226) | 0.199 |
| **p < 0.01 | | |

Discussion

The current study investigated the effects of air pollutants on the reproductive health of men. Our interest in the topic was fueled by the limited studies and controversies on the effects of toxic ambient particles on the reproductive system.

As air pollutants are hazardous to humans, it is infeasible and unethical to conduct an interventional study on patients. Most studies on the effects of ambient pollutants on humans were retrospective or observational and only focused on the effects of pollutants on semen quality.

Zhou et al. reported that urban areas had high SO₂ and NO₂ concentrations and that they were associated with proportionately few sperms with normal morphology. Liu et al analyzed exposure–response relationships of 2841 semen samples and found that SO₂ exposure was associated with a decreased semen concentration and total sperm count.^{17, 18} In addition, Broggia et al. reported that workers occupationally exposed to NO₂ had significantly lower total sperm motility than those not exposed to NO₂.¹⁹ However, Zhang et al. found that SO₂, NO₂, and PM2.5 were not associated with altered semen quality.²⁰

The effect of ozone on the reproductive health of men was relatively higher than that of other ambient pollutants. Zhang et al. obtained the air pollutant data from the national real-time platform for city air quality monitoring and found high ozone exposure to be an independent factor predicting low semen concentrations.²⁰ Farhat et al. reported compromised semen quality after exposure to a high O₃ level in 28 patients with systemic lupus erythematosus.¹⁰ Echoing the findings of the aforementioned studies, Sokol et al. analyzed 5134 semen samples and found a negative association between ambient O₃ exposure and sperm concentration.²¹ In addition, Wdowiak et al. found that ozone reduces the percentage of sperms with normal morphology.²² Nevertheless, Hansen et al. found no significant effect of O₃ on sperm quality in fertile men.²³

Our study found that NO₂ exposure was negatively associated with sperm concentration and sperm motility, which is consistent with the finding of Broggia.¹⁹ Conversely, O₃, SO₂, and PM_{2.5} had no statistically significant effects on semen quality.

The detailed mechanism in which the ambient pollutants interfere with the male reproductive system was not well elucidated. One possible mechanism is induction of oxidative stress in the reproductive system. After exposing a mice model to a high PM_{2.5} concentration, a decrease was observed in superoxide dismutase (SOD), which is one of the crucial enzymes for protecting cells from reactive oxygen species (ROS). Furthermore, an increase in heme oxygenase (HO) was observed. HO plays a role in metabolizing ROS, serving as an indicator of oxidative stress. These changes were observed in testicular biopsy, signifying that ambient pollutants can alter the oxidative balance in the testicular microenvironment.¹³ Mirowsky et al. isolated primary human bronchial epithelial cells in a culture medium and exposed the cells to different levels of NO₂ and O₃. The expression of oxidative-stress-related gene, especially heme oxygenase (decycling) 1 (HMOX₁), which encodes HO, significantly increased in a dose-dependent manner. Additionally, the expression of proinflammatory-related gene, including interleukin-6 and interleukin-8, increased with NO₂ and O₃ exposure, which supports the pro-oxidative stress role of those ambient pollutants.²⁴

Furthermore, SO₂ plays a role in altering oxidative stress. Meng et al. demonstrated that after exposing mice to a high concentration of SO₂, the level of antioxidant enzymes, including glutathione peroxidase (GPx) and SOD, decreased significantly compared with the control group.¹¹ Moreover, ozone plays a role in increasing oxidative stress. Exposing a rat model to O₃ and other concentrated ambient particles increased the lactate dehydrogenase level and increased the activities of antioxidant enzymes including SOD and catalase in the cardiopulmonary system, altogether signifying increased oxidative stress. Moreover, ozone penetrates the blood–gas barrier, and ROS circulates around different organ systems, including the reproductive system.^{25, 26}

Spermatozoon is a cell type that generates ROS on its own, which is crucial in the acrosome reaction. However, if the balance between oxidative stress, ROS, and antioxidative capacity is disturbed, it will lead

to reduced sperm quality. Excessive ROS attacks the fluidity of the sperm plasma membrane and induces DNA damage in the sperm nucleus.^{25, 26} In addition, spermatozoa are more vulnerable to oxidative stress than oocytes as they lack downstream enzymes that participate in base excision repair.^{11, 27} Testicular biopsy of a mice model showed an altered expression of HO and SOD after exposure to ambient pollutants, signifying that increased oxidative stress within the reproductive system could be induced by ambient pollutants. HO plays a role in metabolizing ROS, serving as an indicator of oxidative stress.^{28, 29}

Human testicular development mainly occurs during puberty, and different cell types, including Sertoli cells, spermatogonia, and spermatocytes, play a role. During childhood, testicles grow mainly due to the elongation of seminiferous tubules. During puberty, testicles enlarge due to an increase in the diameter of the seminiferous tubules.^{30, 31} Later, the exponential growth of germ cells during spermatogenesis constitutes the increase in the testicular volume.^{30, 32}

The effect of increased oxidative stress on the testicles has been studied in mice models. Li et al. exposed rat models to oxidative stress caused by crotonaldehyde, which is used to produce sorbic acid, a food preservative. The experimental group had significantly lower testicular and epididymal weights than the controls. Furthermore, in the exposed group, the concentration of malondialdehyde (MDA), a direct indicator of lipid peroxidation-induced injury caused by ROS, significantly increased, whereas GPx and SOD activities decreased.³³ Farsani et al. induced oxidative stress within the mouse testicles by using doxorubicin. The MDA level decreased with an increase in GPx and SOD activities. Moreover, after 8 weeks, the testicular volume, epididymal sperm count, and seminiferous tubule diameter decreased in the exposed group compared with the control group.^{34, 35} Balanced ROS within the testicular environment is crucial. Overloaded oxidative stress and excessive lipid peroxidation can lead to germ cell damage and testicular degeneration. Koksai et al. evaluated the association between oxidative stress measured using MDA and the testicular biopsy obtained from infertile men. Thus, the high MDA level was associated with mild to moderate hypospermatogenesis and maturation arrest.^{35, 36}

To our knowledge, this is the first study to evaluate the effects of ambient pollutants on the testicular volume of infertile men. Nevertheless, the current study has several limitations. First, we considered the exposure variation on the basis of daily average exposure concentrations for a specific period (2 years), while ignoring the effects of the peak and exposure of the pollutant within a day. Second, our study was retrospective and observatory in design, as intentionally exposing participants to air pollutants is not ethical. Moreover, our study population was relatively small, and some data were missing. Therefore, the statistical power to detect the effects of pollutants on the reproductive system and sex hormone profile may be weakened. Furthermore, selection bias was inevitable, as most of our patients were from northern Taiwan. Recruiting patients from other parts of Taiwan and future cross-institute research are recommended to strengthen the statistical power. In addition, other potential confounders may exist that are not included in this study. Third, using the residential address of the study population may not correctly represent the exposure level as the exposure level of candidates to pollution when they commute to other places is not considered.

Conclusions

In conclusion, ambient pollutants are negatively associated with male reproductive health of infertile men. Exposure to nitrogen oxides was negatively associated with sperm concentration and sperm motility. Moreover, exposure to SO₂ was negatively associated with the testicular volume. Furthermore, we studied changes in the physical structure, which was not investigated in previous studies. However, the strength of the result is limited by the retrospective nature of the study. Future prospective studies could compare the different exposure levels of pollutants and change in sperm quality and physical examination findings to strengthen the results.

Abbreviations

WHO

world health organization

PM 2.5

particulate matters 2.5

O₃

ozone

SO₂

sulfur dioxide

NO

nitrogen monoxide

NO₂

nitrogen dioxide

BMI

body mass index

SOD

superoxide dismutase

HO

heme oxygenase

ROS

reactive oxygen species

HMOX₁

heme oxygenase (decycling) 1

GPx

glutathione peroxidase

MDA

malondialdehyde

Declarations

Ethics approval and consent to participate

The study design was retrospective and observation study. The institutional review board of the National Taiwan University approved our study and waived the informed consent requirement because of the retrospective design of our study. (the number of IRB: 20190311 RIND)

Consent for publication.

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

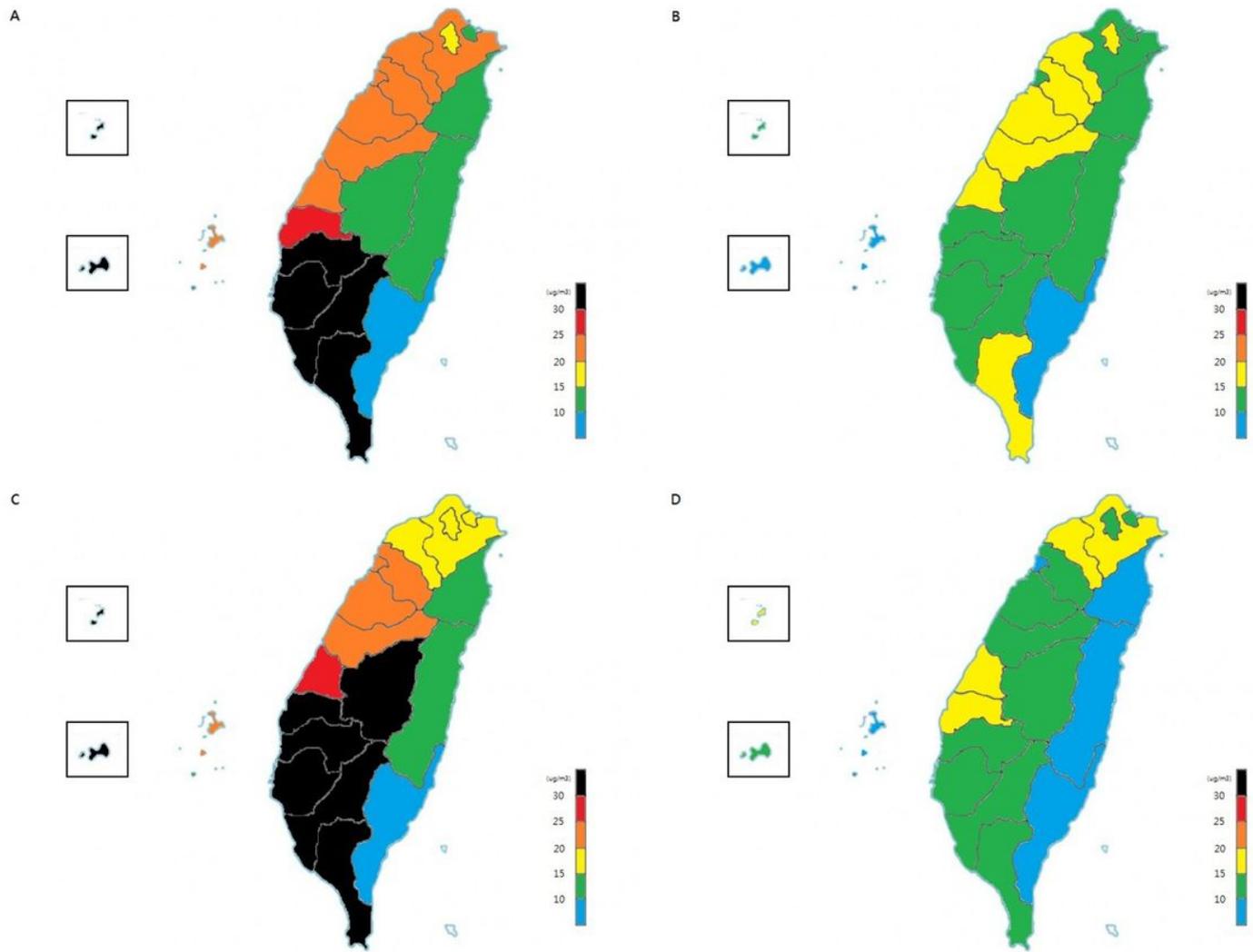


Figure 1

Distributions of particulate matter 2.5 concentration in a: January 2016; b: July 2016; c: January 2017; and d: July 2017 (adapted from the source of wikimedia commons)

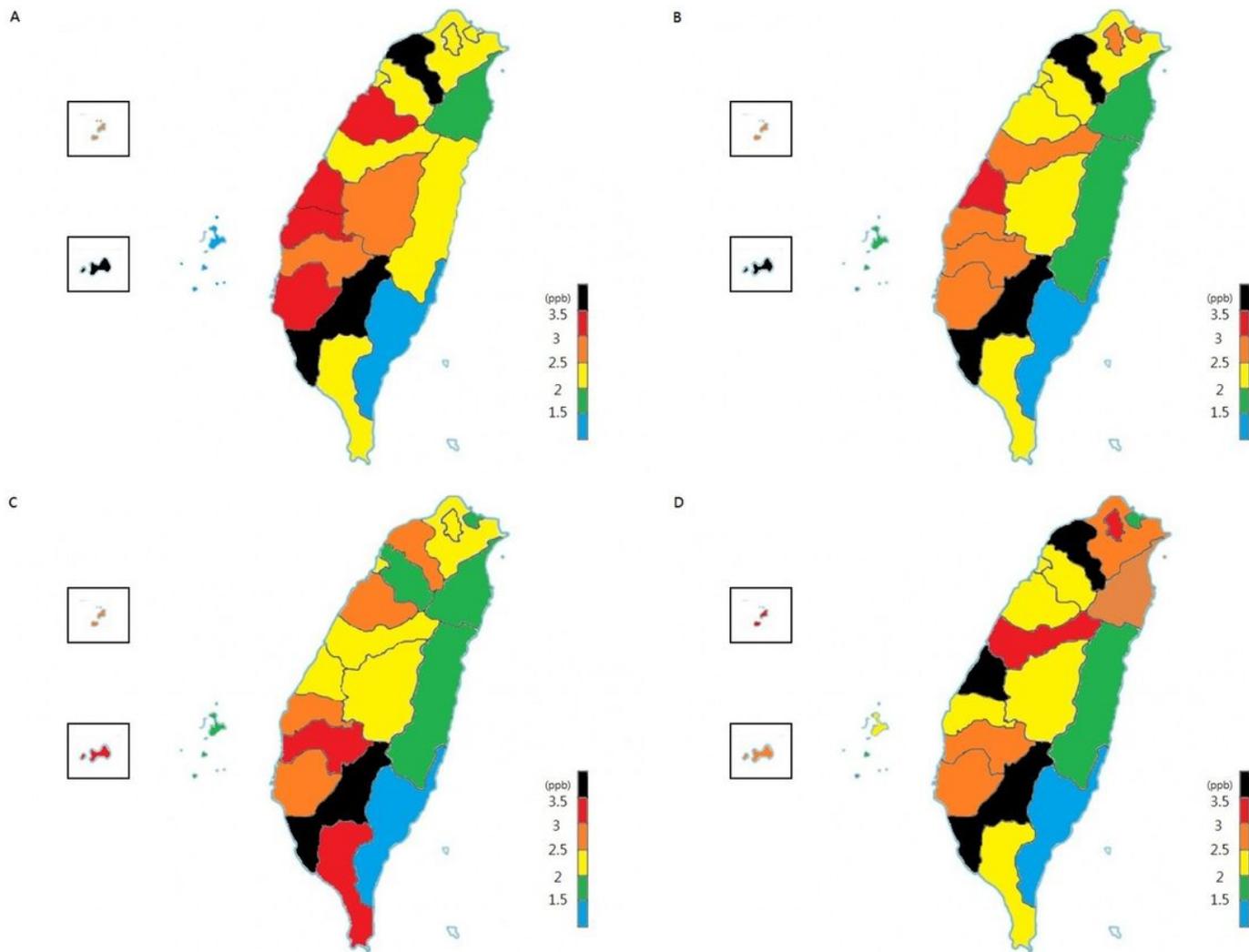


Figure 2

Distributions of sulfur dioxide concentration in a: January 2016; b: July 2016; c: January 2017; and d: July 2017 (adapted from the source of wikimedia commons)

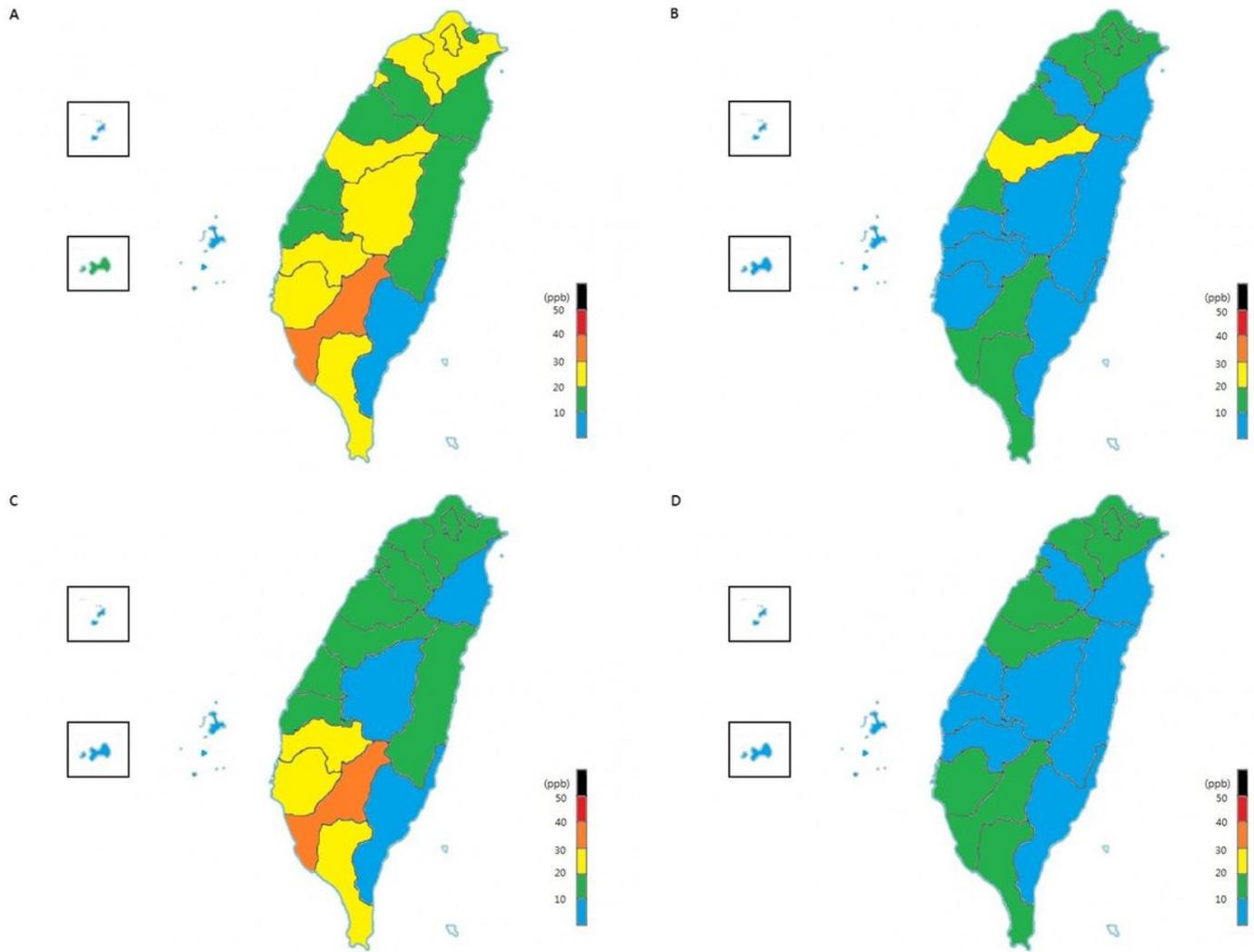


Figure 3

Distributions of nitrogen oxides concentration in a: January 2016; b: July 2016; c: January 2017; and d: July 2017 (adapted from the source of wikimedia commons)

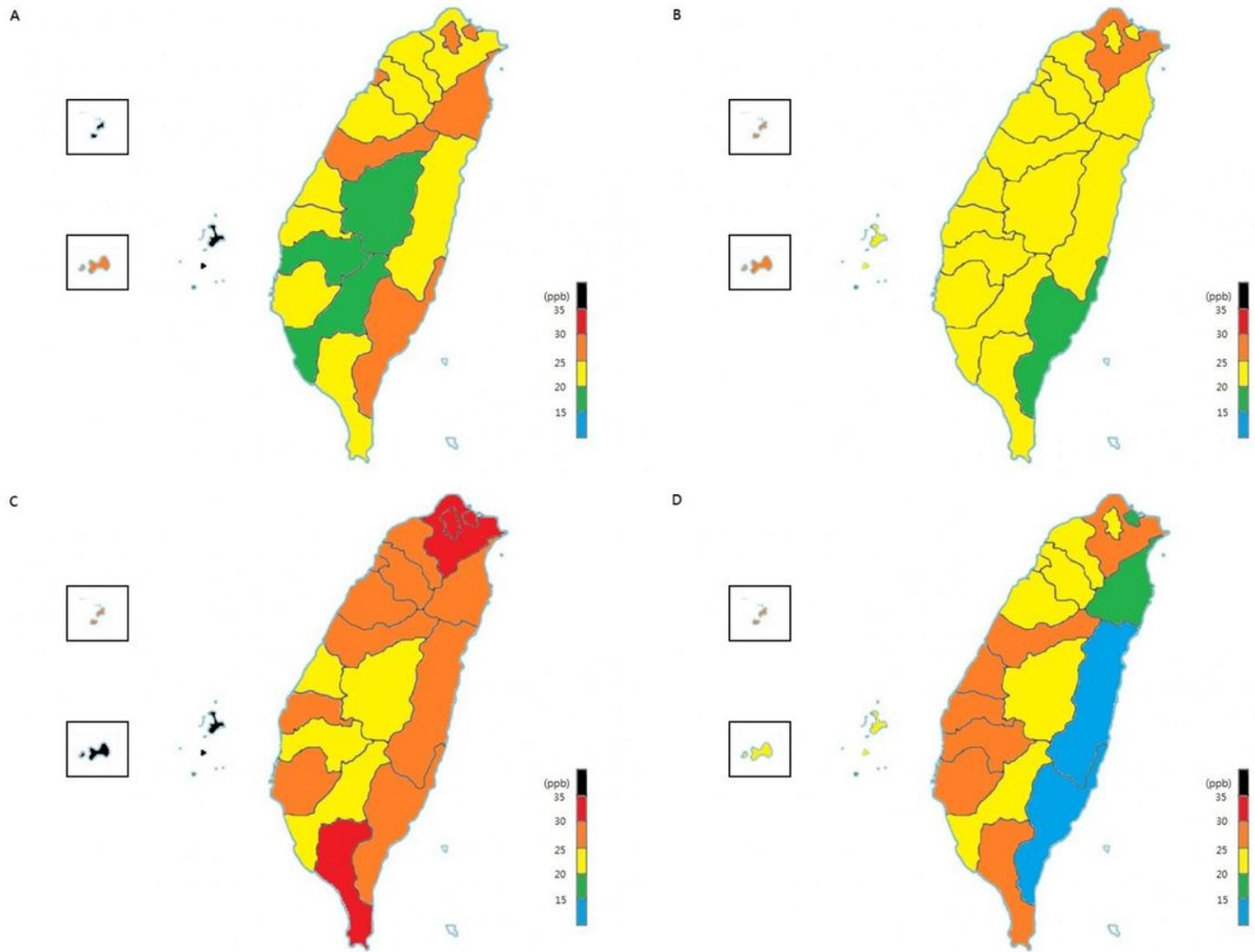
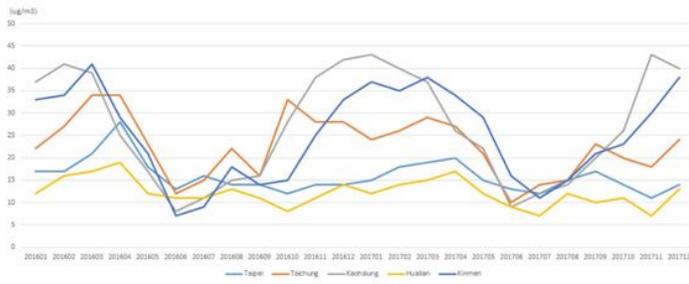


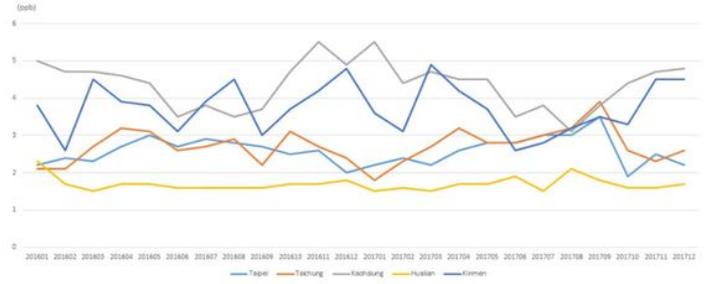
Figure 4

Distributions of ozone concentration in a: January 2016; b: July 2016; c: January 2017; and d: July 2017 (adapted from the source of wikimedia commons)

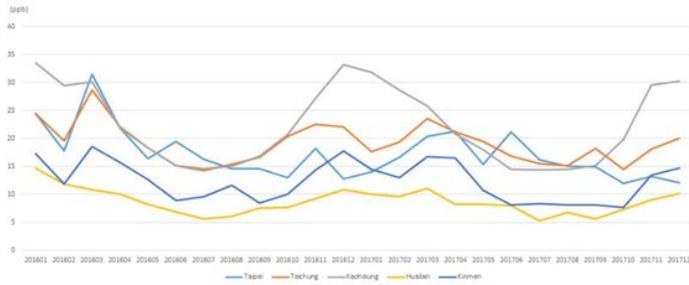
(a)PM_{2.5}



(b)SO₂



(c)NO_x



(d)O₃

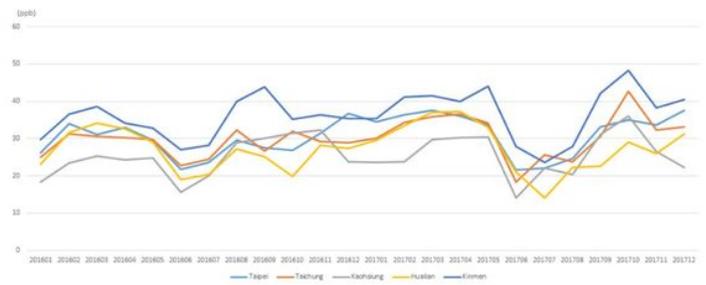


Figure 5

Ambient pollutants concentration in Taiwan from January 2016 to December 2017. a: Particulate matters 2.5; b: Sulfur dioxide; c: Nitrogen oxides; and d: Ozone